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Letter to the Editor

Novel mutations in the pejvakin gene are associated with autosomal recessive non-syndromic hearing loss in Iranian families

To the Editor:

Hearing loss is a very heterogeneous disorder and may be due to genetic or environmental causes, or both. The incidence of pre-lingual deafness is about 1 in 1000 neonates of which more than 60% of cases are inherited (1–3). About 80% of hereditary deafness cases are non-syndromic and the major mode of inheritance is autosomal recessive (4). A novel gene, DFNB59 encoding pejvakin located on chromosome 2q31.2, has been very recently shown to cause neural deafness in four Iranian families. The pejvakin gene is predicted to encode a polypeptide of 352 amino acids containing a nuclear localization signal in residues 249–258 and zinc-binding motif in residues 305–331 (5).

Here, we report mutation analysis for DFNB59 gene in 30 autosomal recessive non-syndromic hearing loss (ARNSHL) families, with an average of five deaf individuals in each family, which also originate from six provinces of Iran. Thirteen families from Chaharmahal va Bakhtiari (southwest), five families from Gilan (north), four families from Khoozestan (southwest), three families from Azerbaijan sharqi (northwest), three families from Kordestan (west) and two families from Tehran (central) were studied. All samples had been previously excluded for mutations in the GJB2 gene (6–11). All patients were clinically characterized and examined for pure-tone audiometry and were found to have mild to profound sensorineural hearing loss. Informed consent was obtained from all subjects or parents of underaged patients.

The DFNB59 gene consists of seven exons, in which the first exon is non-coding. The entire coding region of the DFNB59 gene was polymerase chain reaction amplified and directly sequenced. The resultant sequences were compared with reference sequence NM_001042702 using SEQSCAPE V2.0 software. To date, only two pathogenic mutations (T54I and R183W) in four pedigrees of a cohort of Iranian consanguineous families have previously been reported (5) and the presence of DFNB59 sequence variations in other populations is not yet known. In the current study, five additional DFNB59 sequence variations were identified including two frameshift (c.726delT and c.988delG) and three missense (c.793C>T, c.793C>G and c.874G>A) changes (Table 1, Fig. 1d). Our data indicated that two novel variants (c.726delT and c.988delG), which are predicted to lead to premature termination at codons 248 and 336, respectively, co-segregate with the severe to profound disease phenotype (Fig. 1a–c) and are not found in 100 control

Table 1. DFNB59 sequence variations identified in Iranian deaf families and hearing controls. All novel sequence variations were named following the nomenclature recommendations (17). The frequency of the sequence variations (c.726delT and c.988delG) in the control population was determined using restriction endonuclease digestion of exon 6 (the mutation c.726delT creates a *Bsr*DI restriction site) and direct sequencing of exon 7 of the DFNB59 gene, respectively

Amino acid changes	Nucleotide changes	Number of chromosomes	Number of families	Type of variations	Number of controls ($n = 100$)
p.F242LfsX7	c.726delT	2	1	Frameshift	0
p.R265C	c.793C>T	3	3	Missense	6
p.R265G	c.793C>G	4	4	Missense	17
p.G292R	c.874G>A	2	1	Missense	1
p.V330LfsX7	c.988delG	2	1	Frameshift	0

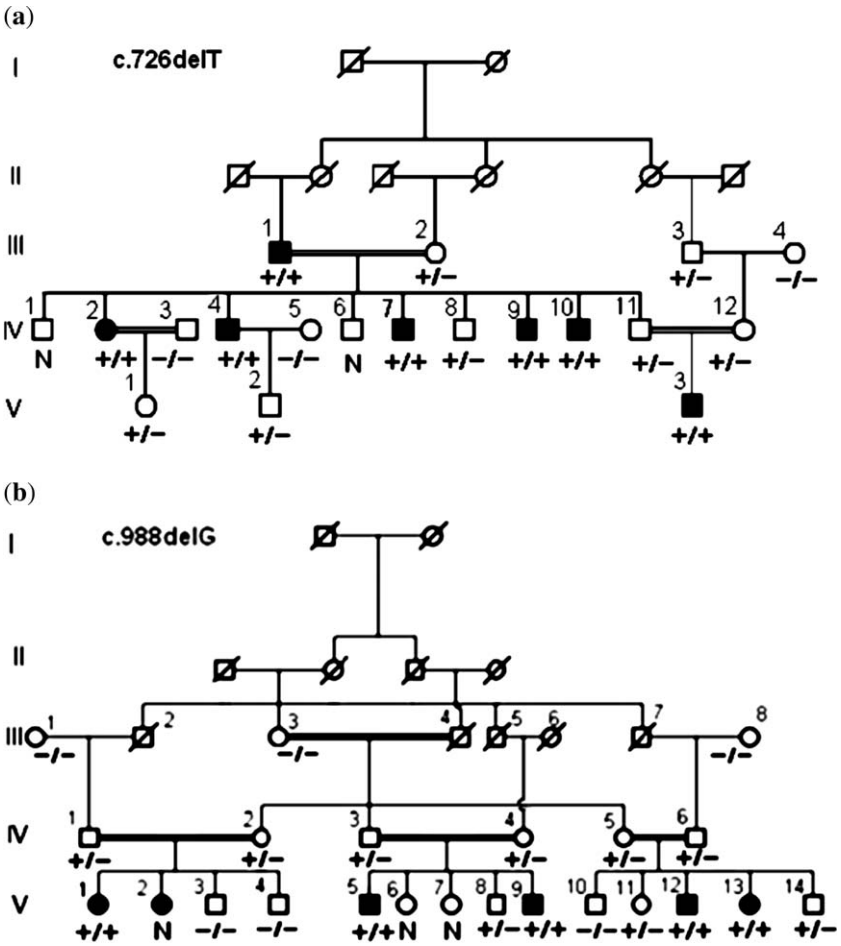

Fig. 1. (**a**) Co-segregation of c.726delT mutation in a family from Gilan. '+' represent the mutant chromosome, '−' represent the wild type and 'N' indicates DNA unavailable. (**b**) Co-segregation of c.988delG mutation in a family from Chaharmahal va Bakhtiari. (**c**) Mean audiometric hearing thresholds for five tested members (III-1, IV-2, IV-4, IV-7 and V-3) of family 'a' (dark squares) and five tested members (V-1, V-5, V-9, V-12 and V-13) of family 'b' (pale triangles). The mean pure-tone averages of thresholds for air conduction at frequencies 0.5, 1 and 2 kHz (the region most critical in speech perception) are 92.8 ± 15.7 dB for family 'a' and 100 ± 13.2 dB for family 'b'. (**d**) Nucleotide sequences of three different novel variants identified in this study. Electropherograms: wild-type sequences are represented at top, heterozygous (gene carriers) in the middle and affected or homozygous individuals at the bottom.

subjects, suggesting a pathogenic role of the abovementioned variations. Another novel missense substitution (c.874G>A) does not occur in a conserved amino acid and does not co-segregate with deafness (data not shown). This change was also found in 1/100 control individuals, suggesting that it is most likely to represent a rare polymorphism and not a pathogenic variation (12–14). The changes involving nucleotide 793 represent known polymorphisms.

Auditory neuropathy is a sensorineural disorder identified by normal otoacoustic emissions (OAEs), but absent or abnormal auditory brainstem responses (ABR). The audiological evaluation of family 'b' (V-5, V-9 and V-12) showed absent ABR with no OAEs (data not shown), which strongly suggests hearing loss of cochlear origin (15). These findings do not meet the diagnostic criteria for auditory neuropathy (5), indicating different audiological manifestation of DFNB59 gene mutations.

Our previous studies indicate that mutation of GJB2 gene accounts for 18.29% of ARNSHL families in Iran (16). While the frequency of DFNB59 mutation in other populations remains to be determined, our data represented here indicate an important association of this gene with ARNSHL in Iran and suggest that it is likely to account for disease in ~6.7% of GJB2-negative families.

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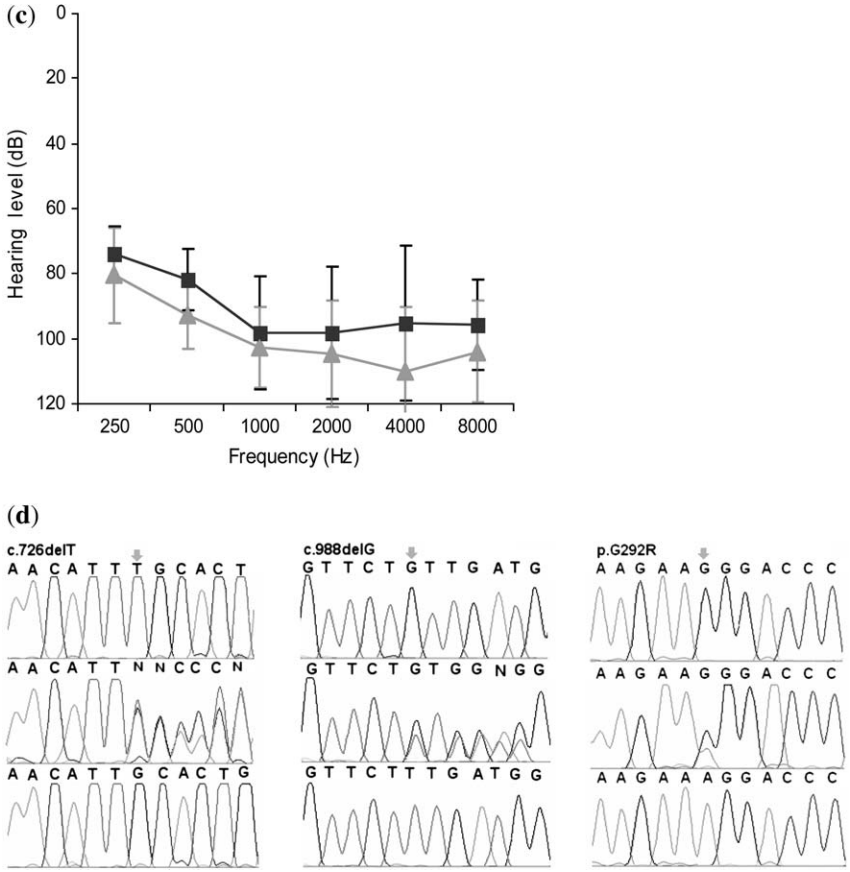
Fig. 1. Continued.



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